Chlorophyll and Lipid Changes on Germination in the Non-green Spores of Thelypteris dentata[1]

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A number of morphological, biochemical, and cytochemical investigations comparing dormant fern spores with 2-celled germinated spores have been reported. Protein and lipid are the major energy reserves in dormant spores. Changes in storage protein (Towill & Ikuma, 1975), protein bodies (Gantt & Arnott, 1965), and lipid (Robinson et al., 1973; Towill & Ikuma, 1975; Gemmrich, 1977) have been investigated during germination. However, most of these investigations have utilized a few species of taxonomically unrelated ferns which have chlorophyll-containing dormant spores (Lloyd & Klekowski, 1970). Proof of the presence of chlorophyll has been shown by the absorption spectra of intact *Onoclea sensibilis* spores (Towill & Ikuma, 1973) and by extraction of *Polypodium vulgare* spores (Robinson et al., 1973). Lloyd and Klekowski (1970) have shown that chlorophyll-containing fern spores are characterized by short viability and a relatively rapid germination rate.

A second—but much more widespread—type of fern spore, believed to be non-chlorophyllous, has remained largely unstudied. This type of spore is characterized by an absence of green pigmentation, long viability and a slow germination rate. The only evidence that non-green fern spores are devoid of chlorophyll is that they lack green pigmentation when observed in the light microscope (Lloyd & Klekowski, 1970).

This investigation was undertaken to determine if the non-green spores of *Thelypteris dentata* contain chlorophyll or lipids associated with the photosynthetic apparatus. Chlorophyll content, lipid classes, and fatty acid compositions of the dormant and germinating spores are compared.

MATERIALS AND METHODS

Fronds of *Thelypteris dentata* (Forssk.) E. St. John were collected from greenhouse-grown plants and placed abaxial surface down on paper. Spore release occurred within two hours. Spores were sifted through lens paper to remove sporangial debris. 200 mg of spores were surface sterilized with an 0.5-1% commercial bleach solution and sown on sterile, modified Knop's medium (Gantt & Arnott, 1965). Germinated spores consisting of a prothallial and rhizoidal cell were obtained in 4 days under continuous fluorescent light (ca. 1500 lux) at about 25°C.

Dormant spores weighing 200 mg, or the germinated spores derived from 200 mg of spores, were homogenized dry in a ground-glass tissue grinder for 5 minutes, moistened with water for 5 minutes, and homogenized with chloroform-

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[1]This study represents a portion of the author's Ph.D. thesis and was carried out at the Hormel Institute, Austin, MN with the technical assistance of J. L. Gellerman and W. H. Anderson. Financial support was provided by U. S. Public Health Service Grant AM 05165 from the N.I.H. to Dr. H. Schlenk. I thank my adviser, Dr. David J. McLaughlin for helpful discussions and suggestions.

methanol (2:1, v/v) for 5 minutes. After filtration, the residue was extracted again with 2:1 chloroform-methanol. Re-extraction of the residue with chloroform-methanol (1:1, v/v) was carried out two times to insure complete extraction of phospholipids. Filtrates were combined and purified according to the methods of Folch et al. (1957). The chloroform phase of the extract was evaporated to dryness under vacuum. Methanol was repeatedly added to the residue and re-evaporated to remove traces of water. The lipid yield of dormant spores was determined at dryness in tared glass tubes.

Thin-layer chromatography was used to identify and estimate the amounts of lipid classes present in spores. Reference compounds, solvents, spray reagents, and techniques were the same as those described by Gellerman et al. (1972). Known quantities of standards were compared with known quantities of samples (400 μg) to estimate the amounts of the lipid classes present.

Techniques used to determine the fatty acid composition of spores including saponification, esterification, preparative thin-layer chromatography of the lipids to remove pigments, and gas-liquid chromatographic analysis are described elsewhere (Gellerman et al., 1972). Results were checked against reference materials of known composition. Identifications and quantifications of methyl esters were made by measuring equivalent chain lengths and peak areas from gas-liquid chromatography.

Chlorophyll content relative to the amount of lipid present (weight per cent lipid) was determined spectrophotometrically at 652 μm (Bruinsma, 1961).

RESULTS AND DISCUSSION

The fresh dormant *Thelypteris dentata* spores contain approximately 50% lipophilic material. Most of the lipid is located in large circular lipid droplets that dominate the cytoplasm (Seilheimer, 1975). The large relative amount of tri-

TABLE 1. LIPID CLASSES AND CHLOROPHYLL CONTENT OF DORMANT AND GERMINATING SPORES OF *Thelypteris dentata*.

Lipid Classes	*Spores (% Lipid)*	
	Dormant	*Germinating*
Neutral Lipids		
Triglycerides	60%	40%
Sterols and Diglycerides (free)	—	—
Carotenes, Squalene, Wax, and Esters	—	—
Glycolipids		
Monogalactosyl Diglyceride	trace	3.5%
Digalactosyl Diglyceride	trace	2.0%
Sulfolipids	—	1.5%
Phospholipids		
Phosphatidyl Glycerol	—	0.2%
Phosphatidyl Choline	0.2%	1.2%
Phosphatidyl Inositol	trace	0.5%
Chlorophyll (weight % lipid)	—	1.1%

glycerides (*Table 1*) present in dormant spores is presumably located in lipid droplets. Triglycerides have been reported as major energy reserves in other fern spores (Robinson et al., 1973; Gemmrich, 1977), moss spores (Karunen, 1971; Gellerman et al., 1972), and certain seeds (Appelqvist, 1975).

The fatty acid of dormant spores consisted primarily of palmitic, oleic, and linoleic acids *(Table 2)*. The most abundant fatty acids of spores are believed to be components of the triglycerides similar to what was reported in spores of *Polypodium vulgare* (Robinson et al., 1973) and *Anemia phyllitidis* (Gemmrich, 1977).

Traces of the glycolipids, monogalactosyl diglyceride, and digalactosyl diglyceride were detected in the dormant spores. The dormant spores also contained a trace of phosphatidyl inositol and a low relative amount of phosphatidyl choline *(Table 1)*. Phosphatidyl choline has been reported in envelope membranes of proplastids (Leese & Leech, 1976) and in mitochondria (Schwertner and Biale, 1973). This finding agrees with the ultrastructural observation of proplastids and mitochondria in the dormant spores (Seilheimer, 1975).

TABLE 2. FATTY ACID COMPOSITION OF TOTAL LIPID EXTRACTS FROM DORMANT AND GERMINATING SPORES OF *Thelypteris dentata*.

Fatty Acids	*Spores (% of Total Fatty Acids)*	
	Dormant	*Germinating*
Palmitic (16:0)	18.7	20.0
Palmitoleic (16:1)	0.3	0.6
Stearic (18:0)	3.8	4.6
Oleic (18:1)	46.9	45.0
Linoleic (18:2)	28.5	27.5
γ -linolenic (18:3 ω 6)	1.1	1.2
Linolenic (18:3 ω 3)	0.2	1.8
Behenic (22:0)	trace	0.1
Arachidonic (20:4 ω 6)	0.2	0.6
Lignoceric (24:0)	trace	0.2

No chlorophyll was detected in the dormant spores of *T. dentata (Table 1)*, nor have chloroplasts been reported in the cytoplasm (Seilheimer, 1975). Furthermore, no significant amounts of lipids associated with chloroplasts, such as monogalactosyl diglyceride, digalactosyl diglyceride, or phosphatidyl glycerol (Leese & Leech, 1976), were detected in the dormant spores *(Table 1)*. Analysis of non-green spore lipids from the fern *Anemia phyllitidis* also has shown a lack of diglycerides (Gemmrich, 1977). These results differ from those reported for the dormant spore of *Polypodium vulgare,* where chlorophyll, phospholipids, and glycolipids, which are normally associated with chloroplasts, are present (Robinson et al., 1973).

Triglycerides *(Table 1),* presumably composed of palmitic, oleic, and linoleic fatty acids *(Table 2),* were also major components of the germinated spores. Similar results were reported in studies of lipid in other germinating fern spores (Robinson et al., 1973; Gemmrich, 1977). This finding suggests that large quantities of lipid reserves remain unutilized during the germination process. However, a decline in triglyceride level was observed in 12 to 15 day old, multicellular gametophytes of *Polypodium vulgare* (Robinson et al., 1973) and *Anemia phyllitidis* (Gemmrich, 1977).

Chlorophyll was present in the germinated spores *(Table 1)*, as were significant relative amounts of glycolipids and phospholipids *(Table 1)* that form structural components of chloroplasts and mitochondria. Monogalactosyl diglyceride and digalactosyl diglyceride are associated with grana formation in chloroplasts

(Leese & Leech, 1976) and chloroplast envelopes (Bahl et al., 1976). Their presence is also reported in mitochondria (Schwertner & Biale, 1973). Sulfolipids are found in chloroplast envelopes, stroma, and grana (Bahl et al., 1976). Phosphatidyl glycerol is a major component of chloroplast thylakoid membranes (Leese & Leech, 1976). Phosphatidyl choline is reported in mitochondria (Schwertner & Biale, 1973) and chloroplasts (Leese & Leech, 1976). The only significant change in the fatty acid composition during germination of *T. dentata* was an increase in linolenic acid *(Table 2)*. Linolenic acid is a major fatty acid of photosynthesizing tissue and probably is a structural element of the chloroplast (Hitchcock & Nichols, 1971).

This investigation of *T. dentata* substantiates the light microscopic observations and comments of Lloyd and Klekowski (1970) that the non-green, dormant spores of ferns lack both chlorophyll and lipid compositions associated with the photosynthetic apparatus. Chlorophyll and lipid composition indicative of chloroplasts were observed after spore germination.

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